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Peripheral participation of the phosphodiesterase 3 on formalin-evoked nociception

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Abstract

The local peripheral (subcutaneous) injection of phosphodiesterase 3 inhibitor trequinsin dose-dependently enhanced formalin-evoked flinching during late second phase of this test. Treatment with the nitric oxide synthase inhibitor *N*-L-nitro-arginine methyl ester or guanylyl cyclase inhibitor 1-*H*-[1,2,4,]oxadiazolo[4,3-a]quinoxalin-1-one significantly reversed trequinsin-induced pronociceptive effect. Results suggest that the peripheral phosphodiesterase 3 may play an important physiologic role on inflammatory pain by controlling cyclic AMP levels and therefore the nociceptor threshold.

Keywords: Phosphodiesterase 3; Cyclic AMP; Trequinsin; Formalin test

1. Introduction

The experimental evidence suggests that the dermal and subcutaneous inflammatory hyperalgesia depends of the stimulation of the cyclic AMP-protein kinase A (PKA) pathway (Ferreira and Nakamura, 1979; Taiwo et al., 1989). In contrast, the cyclic GMP-protein kinase G (PKG) pathway has the opposite action (Ferreira and Nakamura, 1979; Cunha et al., 1999). Phosphodiesterases play critical roles in controlling intracellular cyclic AMP and cyclic GMP. Drugs like theophylline, which inhibits phosphodiesterases unspecifically, increase inflammatory hyperalgesia (Ferreira and Nakamura, 1979).

Two major families of peripheral phosphodiesterases have been implicated in nociception, including cyclic AMP-specific phosphodiesterase 4 and cyclic GMP-specific phosphodiesterase 5. Peripheral inhibition of phosphodiesterase 4 increases or prolongs hyperalgesia (Taiwo et al., 1989; Cunha et al., 1999), whereas that peripheral inhibition of phosphodiesterase 5 produces antinociception and enhances the antinociceptive effect of several analgesics (Mixcoatl-Zecuatl et al., 2000). This antinociceptive effect has been related to accumulation of cyclic GMP. The participation of other phosphodiesterases has not been reported, however since phosphodiesterase 3 breakdowns cyclic AMP we have hypothesized that this enzyme could play an important role to control, together with phosphodiesterase 4 and 5 (Cunha et al., 1999), the nociceptor threshold. Therefore, the purpose of the present study was to assess the possible participation of phosphodiesterase 3 on formalin-induced nociception in rats.

2. Materials and methods

2.1. Animals

Experiments were performed on adult female Wistar rats (body weight range, 180–220 g) of 6 to 7 weeks of age. The animals were obtained from our own breeding facilities and had free access to food and drinking water before experiments. All experiments followed the Guidelines on Ethical Standards for Investigation of Exper-

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imental Pain in Animals (Zimmermann, 1983). Additionally, the study was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados, México, DF, México).

2.2. Measurement of nociceptive activity

Nociception was assessed using the formalin test (Dubuisson and Dennis, 1977; Aguirre-Bañuelos and Granados-Soto, 1999). The rats were placed in open Plexiglas observation chambers for 30 min to allow them to acclimate to their surroundings; then they were removed for formalin administration. Fifty μl of diluted formalin (1%) were injected s.c. into the dorsal surface of the right hind paw with a 30-gauge needle. The animals were returned to the chambers and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed in each chamber to enable unhindered observation. Nociceptive behavior was quantified as the numbers of flinches of the injected paw during 1-min periods every 5 min, up to 90 min after injection (Wheeler-Aceto and Cowan, 1991). Flinching was readily discriminated and was characterized as rapid and brief withdrawal, or as flexing of the injected paw. Formalin-induced flinching behavior was biphasic (Dubuisson and Dennis, 1977; Wheeler-Aceto and Cowan, 1991). The initial acute phase (0–10 min) was followed by a relatively short quiescent period, which was then followed by a prolonged tonic response (15–90 min). Phase 2 was further divided in phase 2A (15–40 min) and 2B (45–90 min) according to Malmberg and Yaksh (1992). At the end of the experiment the rats were sacrificed in a CO_2 chamber.

2.3. Drugs

Trequinsin, *N*-L-nitro-arginine methyl ester (L-NAME), *N*-D-nitro-arginine methyl ester (D-NAME) and 1H-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one (ODQ) were purchased from RBI (Natick, MA, USA). Trequinsin, L-NAME and D-NAME were dissolved in saline. ODQ was dissolved in dimethyl sulfoxide (10%). All other reactants were of analytical grade.

2.4. Study design

Rats received 50 μl of vehicle (saline) or increasing doses (1, 5, 10 and 15 μg/paw in 50 μl) of trequinsin co-injected with formalin (1%) into the dorsal surface of the right hind paw. To determine whether trequinsin acted locally, it was administered to the left (15 μg, contralateral) paw at the same time that formalin was injected into the right paw, and the corresponding effect on nociceptive behavior was assessed. To determine whether trequinsin-induced peripheral pronociception was mediated by the nitric oxide (NO)-cyclic GMP pathway, the effect of treatment (10 min post-treatment) with the appropriate vehicle (10% DMSO for ODQ and saline for L-NAME and D-NAME) or L-NAME (100 μg/paw), ODQ (100 μg/paw) and D-NAME (100 μg/paw) on trequinsin (15 μg/paw)-induced pronociception was assessed. Each rat received 2 injections and appropriate controls for the injection and vehicles were performed before starting the formal study. Doses and drug administration schedule of L-NAME and ODQ were selected based on previous reports (Mixcoatl-Zecuatl et al., 2000) and on pilot experiments in our laboratory. Rats in all groups were tested for possible side effects observed as a reduction of righting, stepping, corneal and pinna reflexes as previously described (Malmberg and Yaksh, 1992).

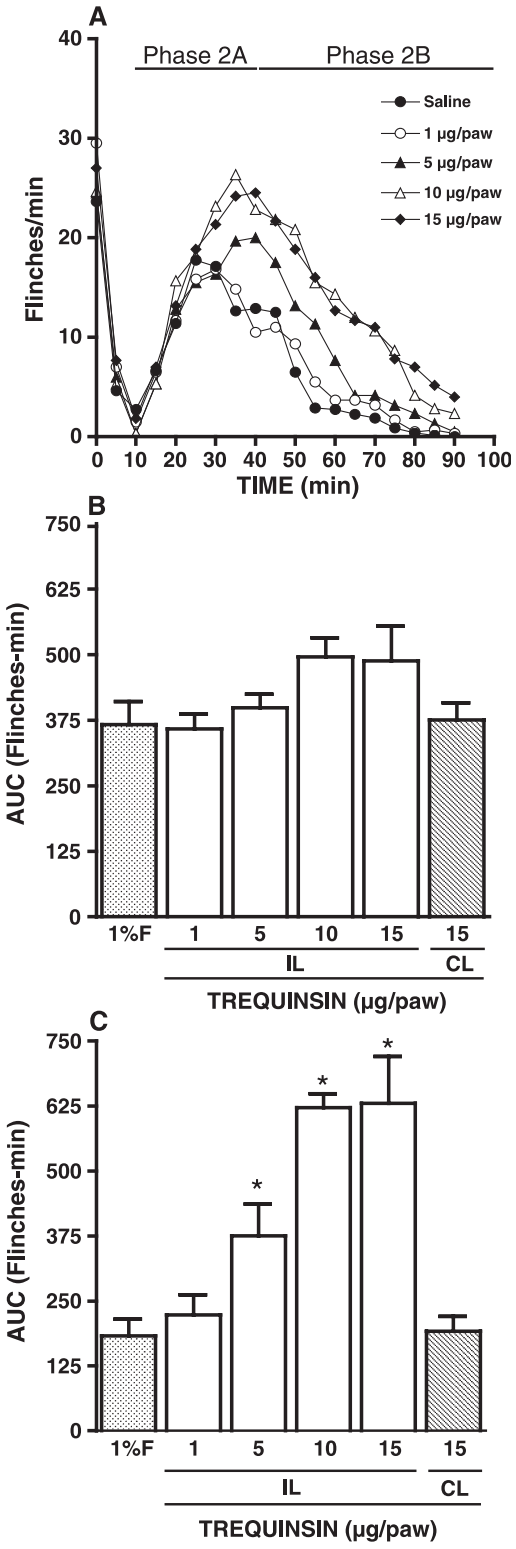

Fig. 1. Pronociceptive effect observed after co-administration of increasing doses of trequinsin and formalin (1%). Pronociceptive effect of trequinsin during both phase 1 and 2 (A), phase 2A (B) and 2B (C) of the formalin test. Data are expressed as the area-under-the-number-of-flinches-against time curve (AUC, panel B and C). IL, ipsilateral; CL, contralateral. Data are the mean ($n=8$) ± S.E.M. Standard errors were omitted for clarity in panel A. * Significantly different from the formalin group (1% F) ($P<0.05$), as determined by analysis of variance followed by Tukey's test.

2.5. Data analysis and statistics

All experimental results are given as the mean±S.E.M. of 8 animals per group. Curves were constructed plotting the number of flinches as a function of time. The area under the number of flinches against time curves (AUC), an expression of the duration and intensity of the effect, was calculated by the trapezoidal rule [(AUC for phase 1 (0–10 min), phase 2A (15–40 min) and phase 2B (45–90 min)]. Division of phase 2 in 2A and 2B was based on the work of Malmberg and Yaksh (1992). Analysis of variance (ANOVA), followed by Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when $P<0.05$.

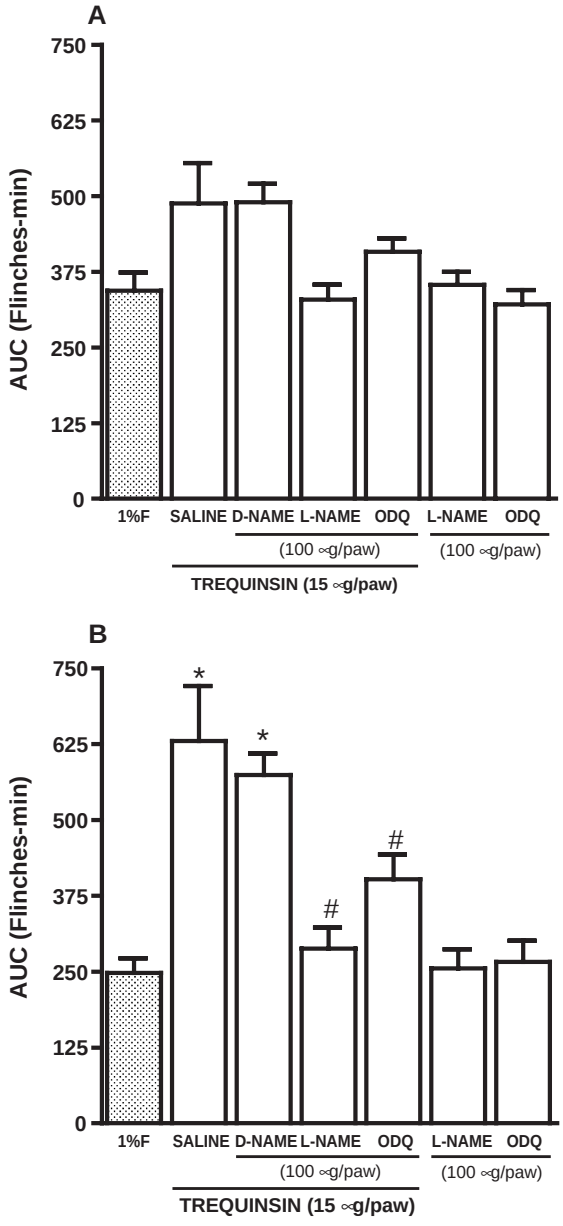

Fig. 2. Effect of *N*-L-nitro-arginine methyl ester (L-NAME) and 1H-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one (ODQ) on the local peripheral pronociceptive effect produced by trequinsin on phase 2A (panel A) and 2B (panel B) of the formalin test. Rats received a local peripheral treatment of trequinsin (15 μg) co-injected with formalin 1% (50 μl) followed by injection of L-NAME or ODQ 10 min later. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the means±S.E.M. of 6 animals. * Significantly different from the formalin group (1% F) ($P<0.05$) and # significantly different from the trequinsin group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

3. Results

Subcutaneous formalin (1%) injection into the right hind paw produced a typical pattern of flinching behavior characterized by a biphasic time course (Fig. 1A). Phase 1 of the nociceptive response began immediately after formalin administration and then declined gradually in approximately 10 min. Phase 2 began about 15 min after formalin administration and lasted about 90 min (Fig. 1A; Dubuisson and Dennis, 1977). Co-injection of the phosphodiesterase 3 inhibitor trequinsin with formalin significantly and dose-dependently increased flinching behavior during phase 2B (Fig. 1A and C), but not phase 2A (Fig. 1B). Nociceptive behavior in phase 1 was unaltered (Fig. 1A). Contralateral administration of trequinsin did not affect formalin-induced nociceptive behavior (Fig. 1B and C).

Local peripheral treatment with L-NAME and ODQ did not produce any effect on formalin-induced nociception (Fig. 2A and B). However, both drugs, but not D-NAME or saline, significantly blocked trequinsin-induced pronociceptive effect to values similar to that observed in the formalin group during phase 2B (Fig. 2B), but not in phase 2A (Fig. 2A).

4. Discussion

In the present study the phosphodiesterase 3 inhibitor trequinsin enhanced formalin-evoked flinching behavior during the phase 2 (particularly phase 2B) of this test. In addition, the nitric oxide synthase inhibitor L-NAME and the guanylyl cyclase inhibitor ODQ (Garthwaite et al., 1995) were able to diminish the pronociceptive effect of trequinsin. Trequinsin is a highly specific phosphodiesterase 3 inhibitor (Lal et al., 1982; Liu and Maurice, 1998). In addition, phosphodiesterase 3 reduces cyclic AMP levels. Therefore, our data suggest that the pronociceptive effect of trequinsin could be due to the inhibition of phosphodiesterase 3 with the consequent increase in cyclic AMP levels. To the best of our knowledge, this is the first report about the pronociceptive effect of trequinsin. These results agree with previous observations showing that peripheral inflammatory hyperalgesia results from activation of cyclic AMP/PKA pathway (Taiwo et al., 1989; Taiwo and Levine, 1991; Aley and Levine, 1999). In addition, the inhibition of another cyclic AMP-hydrolyzing phosphodiesterase (i.e., phosphodiesterase 4) significantly increased carragenaan- or prostaglandin E_2-evoked mechanical hyperalgesia (Taiwo et al., 1989; Cunha et al., 1999). Moreover, it has been proposed that the serotonin- or PGE_2-mediated increase in excitability in sensory neurons may be due to the cyclic AMP-PKA-dependent modulation of the tetrodotoxin-resistant sodium

channel (England et al., 1996; Cardenas et al., 2001). Therefore, our results suggest a significant participation of peripheral phosphodiesterase 3 to maintain the nociceptor threshold by controlling the levels of cyclic AMP induced by several hyperalgesic agents. This suggestion is in line with evidence showing that phosphodiesterase 3 is expressed in several tissues (Beavo, 1995; Liu and Maurice, 1998).

L-NAME and ODQ significantly reduced the pronociceptive effect of trequinsin, suggesting the participation of the NO-cyclic GMP pathway on trequinsin-induced pronociception. This result is according with previous observations showing that peripheral administration of NO synthesis inhibitors is able to reduce bradykinin- or PGE_2-induced hyperalgesia (Nakamura et al., 1996; Aley et al., 1998). In addition, L-arginine, but not D-arginine, significantly increases nociceptive behavior induced by formalin (Kawabata et al., 1994), suggesting a pronociceptive role for NO. These data also suggest that formalin administration may lead to an increase of NO (Rivot et al., 2002) which in turn would lead to formation of cyclic GMP by activation of the guanylyl cyclase. The NO-cyclic GMP pathway has been associated with both pronociceptive (Kawabata et al., 1994; Nakamura et al., 1996; this study) and antinociceptive (Sachs et al., 2004) effects. These opposite effects have been related with the type and intensity of the noxious stimuli, rat strain and particularly with the dose or concentration reached at the active site. In this sense, evidence suggests that low doses are associated with antinociception, whereas that medium or high doses of NO or cyclic GMP produce nociception (Prado et al., 2002; Tegeder et al., 2002). More recently it has been proposed that differences in peripheral studies could be due to the site of administration as intradermal, but not subcutaneous, injection of NO and cyclic GMP donors increased PGE_2-induced nociception (Vivancos et al., 2003). Taken together data suggest that formalin may cause nociception by stimulation of the NO-cyclic GMP pathway in the dermis, while trequinsin produces its pronociceptive effect by inhibiting phosphodiesterase 3 with the consequent increase of cyclic AMP in the subcutaneous tissues.

The possible targets for the NO-cyclic GMP pathway include PKG (Deka and Brading, 2004), potassium channels (Sachs et al., 2004) and phosphodiesterase 3 (Meacci et al., 1992; Beavo, 1995). Among others, activation of PKG and potassium channels has been related with peripheral antinociception (Ortiz et al., 2002; Sachs et al., 2004). The pronociceptive effect of the NO-cyclic GMP pathway could be related with fact that phosphodiesterase 3 enzymes are cyclic AMP-specific phosphodiesterases that are inhibited by cyclic GMP (Meacci et al., 1992). Then, it is likely that the cyclic GMP-induced phosphodiesterase 3 inhibition may contribute, at least in part, to the pronociceptive effect observed after administration of trequinsin.

In conclusion, this study showed that the peripheral administration of the phosphodiesterase 3 inhibitor trequinsin increases formalin-evoked flinching behavior and suggests that cyclic GMP-inhibited cyclic AMP-specific phosphodiesterase 3 plays an important role in maintaining the nociceptor threshold.

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